The role of tissue factor in patients undergoing open repair of ruptured and nonruptured abdominal aortic aneurysms

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Background: **Ruptured abdominal aortic aneurysm (AAA) is associated with the development of a procoagulant and hypofibrinolytic state. Tissue factor (TF) and its naturally occurring inhibitor, tissue factor pathway inhibitor (TFPI), play a central role in the initiation and progression of such a hypercoagulable state, but their role in patients undergoing open AAA repair has not previously been examined.**

Methods: **A prospective study was conducted of 17 patients undergoing elective AAA repair and 10 patients undergoing emergency AAA repair. Blood was taken before induction, and 5 minutes, 24 hours, and 48 hours after aortic cross-clamp release and assayed for plasma TF, TFPI, tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI), and thrombin-activatable fibrinolysis inhibitor (TAFI) activities.**

Results: **TF activity was significantly higher at all time points in patients with ruptured AAA compared with nonruptured AAA. The median (interquartile range, IRQ) TF activity (AU/mL) was 9.9 vs 3.2 (IRQ, 5.9 to 12.6 vs 2.0 to 7.6; $P = .005$) at preinduction; 10.7 vs 1.5 (IRQ, 9.2 to 18.3 vs 0.1 to 6.6; $P = .003$) at 5 minutes after clamp release; 9.5 vs 3.3 (IRQ, 7.0 to 13.5 vs 1.0 to 7.9; $P = .013$) at 24 hours, and 9.6 vs 3.9 (IRQ, 7.6 to 12.6 vs 2.4 to 8.7; $P = .006$) at 48 hours. TFPI levels were not significantly different between ruptured AAA and nonruptured AAA before or during operation but became significantly elevated at 24 and 48 hours in patients who had undergone repair of ruptured AAA. Ruptured AAA repair was associated with a hypofibrinolytic state compared with nonruptured AAA.**

Conclusions: **The present study has demonstrated for the first time, to our knowledge, that ruptured AAA is associated with significantly higher perioperative levels of circulating TF compared with nonruptured AAA. Furthermore, in the immediate perioperative period, the high levels of TF are not associated with a corresponding rise in TFPI levels, indicating an unopposed prothrombotic state. Direct inhibition of TF by administration of anti-TF antibodies or recombinant TFPI remains to be evaluated in subjects presenting with hemorrhage due to ruptured AAA, but if given early enough, it may attenuate the early deleterious effects of unopposed TF expression and ultimately contribute to improved outcomes. (J Vasc Surg 2007;46:682-6.)**

Ruptured abdominal aortic aneurysm (AAA) is associated with the development of a procoagulant and hypofibrinolytic state.[1,2] The most obvious manifestation of this hemostatic derangement is disseminated intravascular coagulation (DIC), which leads to thrombotic and hemorrhagic complications. Disorders of coagulation and fibrinolysis have also been implicated in the pathogenesis of myocardial infarction (MI), multiple organ dysfunction syndrome (MODS), acute respiratory distress syndrome (ARDS), and the systemic inflammatory response syndrome (SIRS), all of which may occur in patients undergoing open AAA repair.[3-5]

Tissue factor (TF) and its naturally occurring inhibitor, tissue factor pathway inhibitor (TFPI), play a central role in the development of these conditions predominantly through their role in the crosstalk between coagulation and inflammation.[5] TF is the most important initiator of intravascular coagulation. After vascular injury, TF is rapidly expressed and triggers coagulation through the activation of factors VII and X. Ultimately TF is inhibited by TFPI,[6] which acts by reversibly inhibiting factor Xa and the factor VIIa/TF complex, thereby limiting thrombin production.

An understanding of the role of TF and TFPI, and their relationship with other markers of coagulation in patients undergoing open AAA repair may point to potential therapeutic targets aimed at reducing morbidity and mortality. The present study compared serial levels of markers of coagulation (TF, TFPI) and fibrinolysis, including tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI), and thrombin-activatable fibrinolysis inhibitor (TAFI), in patients undergoing open repair of ruptured and nonruptured AAA.

METHODS

Patients. After local ethical approval, a prospective study was conducted of 17 patients (15 men and 2 women) with a median age of 72.5 years (range, 60 to 81 years) undergoing elective repair of asymptomatic infrarenal AAA, and 10 patients (9 men and 1 woman) with a median age of 75 years (range, 65 to 86 years) undergoing emergency repair of ruptured AAA. In patients undergoing elective

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Competition of interest: none.

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0741-5214/$32.00

doi:10.1016/j.jvs.2007.05.057

Table I. Clinical and operative data in subjects operated on for nonruptured and ruptured infrarenal abdominal aortic aneurysm

Clinical and operative data	*Nonruptured AAA, median (range) (n = 17)*	*Ruptured AAA, median (range) (n = 10)*	P*
Preoperative			
AAA size (mm)	60 (53-90)	—	
Onset of symptoms to surgery (hr)	—	4.5 (2-12)	
Intraoperative			
Total operation time (min)	195 (85-375)	94 (55-240)	.001
Clamp time (min)	68 (40-150)	64 (35-120)	.625
Measured blood loss (mL)	2450 (1000-6000)	2750 (980-6400)	.886

AAA, Abdominal aortic aneurysm.
*Mann-Whitney *U* test.

AAA repair, the median (range) aortic diameter was 60 mm (range, 53 to 90 mm). All subjects operated on for ruptured AAA had at least one documented episode of hypotension (systolic blood pressure <100 mm Hg) preoperatively, and ruptured AAA was confirmed at operation by the presence of fresh intraperitoneal or retroperitoneal blood. The median delay to surgery from onset of symptoms in patients with ruptured AAA was 4.5 hours.

All subjects underwent repair through a transverse supraumbilical incision with infrarenal clamping. No patient with a ruptured AAA was given systemic heparin; however, those undergoing elective AAA repair received 5000 units of heparin immediately before aortic cross-clamping.

Sample collection and assay methods. After discarding the first 10 mL, arterial blood was sampled from an indwelling radial arterial line immediately before induction of anesthesia (sample A) and 5 minutes (sample B), 24 hours (sample C), and 48 hours (sample D) after aortic clamp release. Samples were placed immediately on ice and centrifuged within 30 minutes of collection at 1400*g* for 30 minutes at 4°C, and the resultant plasma and serum were stored at −80°C for later batch analysis. Commercially available chromogenic assays were used to determine the activity of t-PA (Coatest t-PA, Chromogenix, Möndal, Sweden), PAI (Coaset PAI, Chromogenix), TF (Actichrome TF, American Diagnostica, Greenwich, CT), TFPI (Actichrome TFPI, American Diagnostica), and TAFI (Actichrome TAFI, American Diagnostica). The normal range for each hemostatic marker was determined by the manufacturer of the assay.

Statistical analysis. Data were analyzed using SPSS 11.0.1 (SPSS Inc, Chicago, Ill). Nonruptured AAA and ruptured AAA were compared using the Mann-Whitney *U* test. In subjects who died, data up to the point where they died were used in the data set and analysis. Because the data were not normally distributed, changes in parameters over time were analyzed using the Wilcoxon signed ranks test, and the Spearman rank test was used to correlate the hematologic and clinical variables. A value of $P < .05$ was taken to be statistically significant. More complicated analysis was not performed because we determined that, with the number of subjects in the study, this would not yield much additional information and would only serve to cloud the issue.

RESULTS

Clinical data. All subjects undergoing elective repair of asymptomatic infrarenal AAA survived to discharge from hospital. Of those undergoing repair of ruptured AAA, the mortality rate was 50% (5/10). One patient died intraoperatively of uncontrollable hemorrhage, one patient who developed DIC and was anuric during surgery died of circulatory failure in the theatre recovery room, and three died of MODS and ARDS in the late postoperative period. Clinical and intraoperative details are shown in Table I.

Markers of fibrinolysis. Because we have previously assessed the effect of AAA rupture on the fibrinolytic system, including D-dimer,[1] we elected to only measure t-PA and PAI activity in this present study to allow a reference point to aid in the interpretation of the more novel coagulation factors studied. A comparison of hematologic variables at various time points between ruptured and nonruptured abdominal aortic aneurysms is presented in Table II.

Before induction of anesthesia, t-PA activity (IU/mL) was significantly lower in patients with ruptured AAA, with a median value of 0.12 vs non-ruptured AAA at 0.54 (interquartile range [IQR], 0.08 to 0.41 vs 0.18 to 1.58; $P = .036$). At all other time points, there was no significant difference in t-PA activity between the two groups, and no significant change in t-PA activity levels was observed with time in either group.

PAI activity (AU/mL) was significantly higher before and during operation in patients with ruptured AAA, with a median preinduction value of 36.9 vs nonruptured AAA of 9.9 (IRQ, 25.3 to 38.4 vs 1.3 to 13.2; $P = .002$), and at 5 minutes after clamp release it was 37.5 vs 11.4 (IRQ, 11.9 to 38.8 vs 9.1 to 23.0; $P = .046$). In ruptured AAA repair, PAI activity remained elevated out to 24 hours but then fell significantly at 48 hours compared with preinduction levels (36.9 [IRQ, 25.3 to 38.4] to 13.5 [IRQ, 9.3to 21.0]; $P = .017$). In nonruptured AAA repair, PAI activity was significantly increased at 24 and 48 hours compared with preinduction levels (preinduction: 9.9 [IRQ, 1.3 to 13.2] vs 24 hours: 11.6 [IRQ, 9.7 to 26.4]; $P = .03$; vs 48 hours: 16.3 [IRQ, 11.2 to 29.4]; $P = .006$).

Tissue factor. TF activity was significantly higher at all time points in patients with ruptured AAA vs nonruptured

Table II. Comparison of hematologic variables at various time points between ruptured and nonruptured abdominal aortic aneurysms

Assay (normal range)	*Sample point**	*Nonruptured AAA, median (range) (n = 17)*	*Ruptured AAA, median (range) (n = 10)*	P†
tPA activity (0.2-2.0 IU/mL)	A	0.54 (0.10-2.45)	0.12 (0.06-7.90)	.036
	B	0.75 (0.10-3.69)	0.38 (0.08-7.60)	.312
	C	0.80 (0.10-5.57)	0.35 (0.09-7.30)	.829
	D	0.38 (0.10-1.36)	0.99 (0.15-2.1)	.297
PAI activity (<15 AU/mL)	A	9.9 (0.1-21.7)	36.9 (2.6-38.8)	.001
	B	11.4 (2.8-29.4)	37.5 (0.1-39.3)	.048
	C	11.6 (2.2-32.1)	37.0 (0.1-39.4)	.124
	D	16.3 (0.8-149.2)	13.5 (5.9-35.3)	.570
TF activity (<2 pM)	A	3.2 (0.1-30.0)	9.9 (3.7-20.3)	.005
	B	1.5 (0.1-31.2)	10.7 (6.7-23.9)	.003
	C	3.3 (0.4-17.2)	9.5 (3.1-18.7)	.013
	D	3.9 (1.0-16.7)	9.6 (4.4-19.5)	.006
TFPI activity (0.73-1.27 U/mL)	A	2.1 (0.1-2.8)	1.9 (1.3-2.4)	.187
	B	2.0 (0.9-3.6)	1.9 (1.3-2.8)	.264
	C	1.7 (0.5-3.0)	2.2 (1.6-2.9)	.013
	D	1.9 (0.5-3.0)	2.5 (1.2-2.8)	.006
TAFI levels (12-20 μg/mL)	A	52.9 (16.6-81.5)	49.0 (39.0-77.3)	.863
	B	29.7 (1.0-77.5)	43.2 (20.6-66.0)	.309
	C	37.1 (18.2-100.0)	46.4 (17.7-63.7)	.675
	D	46.9 (1.0-92.0)	46.3 (4.3-76.2)	.537

AAA, Abdominal aortic aneurysm; *TPA,* tissue plasminogen activator; *PAI,* plasminogen activator inhibitor; *TF,* tissue factor; *TFPI,* tissue factor pathway inhibitor; *TAFI,* thrombin-activatable fibrinolysis inhibitor.

* *Sample A,* Immediately before induction of anesthesia; *sample B,* 5 minutes after aortic cross clamp release; *sample C,* 24 hours after aortic cross-clamp release; *sample D,* 48 hours after aortic cross-clamp release.

†Mann-Whitney U test.

AAA. The median (IQR) preinduction TF activity (pM) vs nonruptured AAA was 9.9 vs 3.2 (IRQ, 5.9 to 12.6 vs 2.0 to 7.6; $P = .005$); at 5 minutes after clamp release it was 10.7 vs 1.5 (IRQ, 9.2 to 18.3 vs 0.1 to 6.6; $P = .003$); at 24 hours it was 9.5 vs 3.3 [IRQ, 7.0 to 13.5 vs 1.0 to 7.9; $P = .013$); and at 48 hours it was 9.6 vs 3.9 (IRQ, 7.6 to 12.6 vs 2.4 to 8.7; $P = .006$; Fig). There was no significant change in TF activity observed with time in either group. Amongst the group as a whole, preinduction TF activity levels correlated significantly with preinduction levels of t-PA (Spearman's coefficient [SC], −0.531; $P = .008$) and PAI activity (SC, 0.633; $P = .001$).

Tissue factor pathway inhibitor. TFPI levels were not significantly different between ruptured AAA and nonruptured AAA before or during operation. At 24 and 48 hours postoperatively, however, TFPI levels were significantly higher in subjects who had undergone repair of ruptured AAA. The median (IQR) TFPI activity (U/mL) vs nonruptured AAA at 24 hours was 2.2 vs 1.7 (IRQ, 1.9 to 2.6 vs 1.5 to 2.0; $P = .013$), and at 48 hours it was 2.5 vs 1.9 (IRQ, 2.3 to 2.6 vs 1.1 to 2.3; $P = .006$). TFPI levels in elective AAA repair correlated significantly with preinduction (SC, −0.630; $P = .007$) and 5-minute postclamp (SC, −0.532; $P = .028$) TF activity levels, but no association was observed in the ruptured AAA group.

Thrombin-activatable fibrinolysis inhibitor. No significant difference in TAFI levels was found between ruptured AAA and nonruptured AAA at any time point. Overall however, there was a significant drop in TAFI levels at 5 minutes after aortic clamp release, which remained significantly lower at 24 hours. The median (IQR) preinduction TAFI (μg/mL) value was 52.5 vs 36.7 (IRQ, 39.9 to 64.3 vs 23.4 to 54.9; $P \leq .001$) at 5 minutes postclamp vs 39.1 (IRQ, 28.5 to 52.5; $P = .008$) at 24 hours. Among the nonruptured AAA group, the acute fall in TAFI after

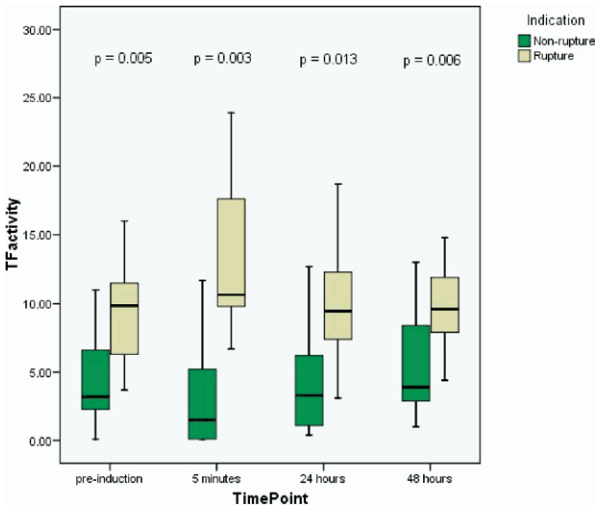

Figure. Serial box plot compares the change in tissue factor *(TF)* activity over time after ruptured and nonruptured abdominal aortic aneurysm repair. The *horizontal line* in the middle of each box indicates the median; the *top* and *bottom borders* of the box mark the *interquartile range*. The *whiskers* mark the standard error.

clamp removal correlated with the change in TF at the same time point (SC, 0.500; $P = .041$).

DISCUSSION

The present study has demonstrated for the first time, to our knowledge, that ruptured AAA is associated with significantly higher perioperative levels of circulating TF compared with nonruptured AAA. Furthermore, in the immediate perioperative period, the high levels of TF are not associated with a corresponding rise in TFPI levels, indicating an unopposed prothrombotic state.

Currently, no published data have examined TF or TFPI in patients with ruptured AAA or compared perioperative changes in these markers between ruptured and nonruptured AAA. Yamazumi et al[7] measured TF levels in patients undergoing elective AAA repair and found no correlation between TF and the other hemostatic markers or aneurysm morphology. TFPI levels showed a significant correlation with thrombin-antithrombin complex, D-dimer, fibrin degradation products, α-2 plasmin inhibitor, and aneurysm size and tortuosity.[7] Nomura et al[8] measured TFPI levels in patients with nonruptured AAA before elective repair and found significantly higher levels compared with age-matched controls.[8]

TF has been measured in trauma patients and in subjects undergoing cardiopulmonary resuscitation after cardiac arrest, populations sharing similar global ischemia and reperfusion characteristics with those with ruptured AAA.[5,9] In both of these groups, TF levels were significantly higher than in control subjects and gradually decreased during a 4-day period.[9] The observed elevations in TF were associated with a reduction in TFPI levels, indicating activation of the extrinsic coagulation pathway without adequate TFPI generation.[10]

In the present study, the difference in TFPI levels between the groups was not significant at induction or 5 minutes after aortic declamping, despite significantly higher TF levels in patients with ruptured AAA. The absence of any correlation between early perioperative TF and TFPI in patients with a ruptured AAA, where a significant negative correlation existed for nonruptured AAA, suggests that an imbalance exists between these two opposing factors in this group of patients. Unlike the previous work on trauma patients, TFPI levels significantly rose at 24 and 48 hours in patients with ruptured AAA. This suggests an early lag phase in the TFPI response, followed by a catch-up phase in an attempt to inhibit the sustained TF response.

This present study confirmed our previous findings that ruptured AAA is associated with a hypofibrinolytic state compared with nonruptured AAA.[1,11] The hypofibrinolytic state persisted to 24 hours in the ruptured AAA group before returning to a similar level as the nonruptured AAA group at 48 hours. TAFI couples the coagulation and fibrinolytic cascades, such that the action of the former down-regulates the activity of the latter.[12] To our knowledge, the present study is the first to assess TAFI levels in patients with AAA.

Despite demonstrating that ruptured AAA was associated with an inhibition of fibrinolysis, we found no significant difference in TAFI levels between the two groups. Similar discrepancies between PAI and TAFI have been observed in subjects with acute coronary syndrome,[13] with differences in weight or different responses to inflammation or shock being implicated. Studies in human sepsis suggest that both PAI and TAFI are responsible for mediating inhibition of fibrinolysis, but PAI appears to be involved early in the septic process, with TAFI responsible for ongoing fibrinolytic inhibition in the later stages.[14]

No significant variations in TAFI levels have been observed between subjects with DIC and controls.[15] In the present study, we observed a significant fall in TAFI levels after aortic declamping, which only returned to baseline values at 48 hours. This acute change in TAFI did not correlate with the other fibrinolytic markers, although the fall in TAFI did correlate with the change in TF levels after aortic declamping, suggesting an inherent link with thrombin production.

What is the clinical value of these findings? Changes in coagulation and fibrinolysis play a major role in the morbidity and mortality associated with critical illness. A hypofibrinolytic state occurring during ruptured AAA repair has been shown to be associated with the development of perioperative myocardial injury.[11] Elevated TF levels after trauma have been associated with DIC, SIRS, ARDS, and MODS.[5] The likely mechanism behind these complications is intravascular and intra-alveolar fibrin deposition; however, it is becoming increasingly clear that coagulation and inflammation are inextricably linked, with inflammation activating coagulation, and vice versa.[16] TF is the principal initiator of coagulation and an important modulator of inflammation, such that an exaggerated or poorly controlled response may contribute to the development of MODS and the higher mortality observed in subjects with a ruptured AAA.

In our study, TF activity preinduction was significantly lower among survivors compared with nonsurvivors; however, this is confounded by the observation that half of subjects with a ruptured AAA died, whereas none in the nonruptured group died, and this finding is therefore more likely to reflect the difference between the two different AAA groups rather than an true association with mortality. The small number of subjects hampered detailed subgroup analysis, and thus it was not possible to assess whether there were any differences in hematologic variables between survivors and nonsurvivors or to assess the impact of other variables such as clamp time, degree of shock, and blood loss.

CONCLUSION

This study has demonstrated that ruptured AAA is associated with an increased TF response that is unopposed by TFPI in the early perioperative period. Because a perioperative hypofibrinolytic state has previously been shown to be associated with myocardial injury, it is possible that this early prothrombotic state may also contribute to the poor outcome seen with ruptured AAA. Direct inhibition of TF by adminis-

tration of anti-TF antibodies or recombinant TFPI has been shown to reduce DIC in sepsis.[6] The effect of this form of intervention in patients presenting with hemorrhage due to ruptured AAA or major trauma remains to be evaluated, but if given early enough, it may attenuate the early deleterious effects of unopposed TF expression and ultimately contribute to improved outcomes.

We would like to thank Professor Jon Deeks, Professor of Health Statistics, University of Birmingham, Birmingham, United Kingdom, for his help with the statistical analysis performed in this study. We also acknowledge Mark Hill, Department of Haematology, Heart of England National Health Service (NHS) Trust for his help with the hematologic assays.

AUTHOR CONTRIBUTIONS

Conception and design: SH, PH, DA
Analysis and interpretation: SH
Data collection: PH, DA
Writing the article: SH
Critical revision of the article: PH, DA, CF, AB
Final approval of the article: SH, PH, CF, AB, DA
Statistical analysis: SH
Obtained funding: AB, DA
Overall responsibility: SH

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Submitted Feb 21, 2997; accepted May 23, 2007.